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Iron Associated Heme-Derivative Biosynthesis from a Metabolic Engineered *Escherichia coli*

Oh-Hee Kwon, Susie Kim, and Pil Kim*

Department of Biotechnology, Catholic University of Korea, Gyunggi 420-743
Tel : +82-2-2164-4964, Fax : +82-2-2164-4865, E-mail : kimp@catholic.ac.kr

Heme is a red-colored prosthetic group of hemoglobin harboring ferrous ion in tetrapyrroles, and has derivatives such as chlorophyll and vitamin B12. Heme is biosynthesized from aminolevulinic acid (ALA) via tetrapyrrole pathway, and ALA is the first committed step in the formation of tetrapyrroles (Fig. 1). 5-Aminolevulinic acid (ALA) itself is a type of amino acid having 5 carbons, which is the first precursor of tetrapyrrole biosynthesis. Based upon the characteristics of the biosynthesis pathway, ALA can be applied to commercial purposes such as agricultural applications (plant growth stimulator, pesticides and insecticides), medical application (anticarcinogenic agents, cancer diagnosis), and biotechnology application (production of cosmetic additives and for the production of heme structured enzymes).

At the current study, the biosynthesis of ALA triggered in *Escherichia coli* through the expression of ALA synthase mediating condensation of succinyl-CoA and glycine. The ALA synthase gene (*hemA*) and the flank region, originated from the photosynthetic *Rhodobacter sphaeroides*, were expressed under control of dual promoter P(lac-trc). The recombinant *E. coli* produced 0.8 g/L and 25.5 mg/L of ALA after cultivation for 24 h in the succinate based medium (S-medium) and in the glucose based medium (G-medium), respectively. To increase the flow of succinyl-CoA that is the substrate of ALA, the NADP-dependent malic enzyme (*maeB*) was co-expressed with ALA synthase (*hemA*). Even though *maeB-hemA* co-expression affected no increase of ALA production in the S-medium, about twice increase of ALA production was found in the G-medium compared with *hemA* expression (39.1 mg/L vs. 25.5 mg/L). Therefore, increase of C4 metabolism via *maeA* expression enabled *E. coli* to supply higher succinyl-CoA, which is consequently linked to the ALA production. Based on the above results, the *maeB-hemA* co-expressing *E. coli* was cultured in the medium containing both of succinate and glucose (GS-medium) to lead more efficient ALA production. Unlike the expectation, the ALA production of *hemA-maeB* coexpressed *E. coli* in GS-medium showed no difference to that of *hemA* single expressed

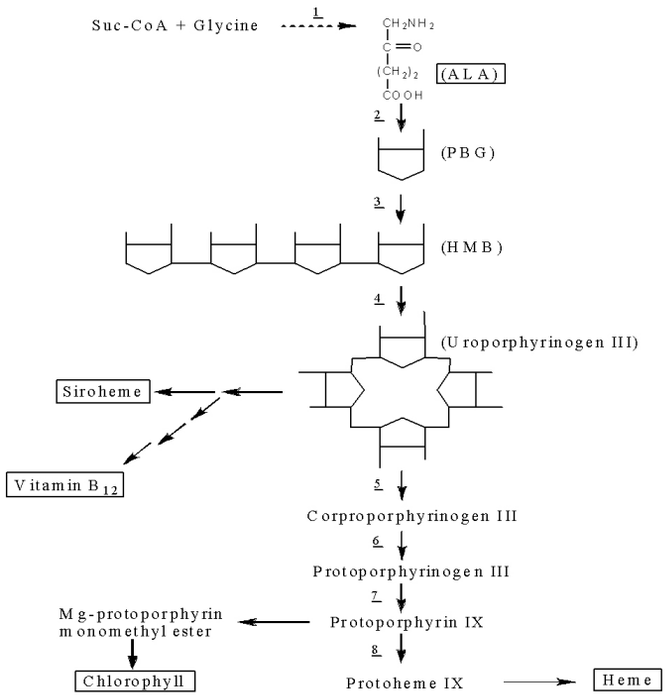

Fig. 1. Biosynthetic pathway for tetrapyrrole. The enzymes are (1) ALA synthase (*hemA*), (2) porphobilinogen synthase (*hemB*), (3) hydroxymethylbilane synthase (*hemC*), (4) uroporphyrinogen synthase (*hemD*), (5) uroporphyrinogen III decarboxylase (*hemE*), (6) coproporphyrinogen III oxidase (*hemF*), (7) protoporphyrinogen IX oxidase (*hemG*), (8) ferrochetolase (*hemH*)

E. coli in G-medium. This result implied *E. coli* in glucose-containing medium might have been attributable to the catabolite repression control which does not transport the secondary carbon source such as succinate. To solve the problem, the artificial control of transporting succinate in spite of the presence of glucose was necessary. The dicarboxylate DAACS transporter (*dctA*) is the membrane protein that transports dicarboxylic acids including succinate. The recombinant *E. coli* expressing tritple genes of *hemA-maeB-dctA* produced 20-folds higher ALA (0.8 g/L) than that expressing *hemA-maeB* in the GS-medium. Further study on co-expression of the pathway to ALA including succinyl-CoA synthase (*SCS*) genes of *sucC* (succinyl-CoA beta chain) and *sucD* (succinyl-CoA alpha chain) for increase the intracellular content of succinyl-CoA is on the way as well as the *maeB* for the stimulation of C4 metabolism and *dctA* for the simultaneous transport control of succinate.

The ALA biosynthesis enhancement led the recombinant *E. coli* cell to accumulate typical red pigment, which is considered the iron-associated heme derivatived from ALA. The red pigment was purified from cell by cold-acid extraction for identification. Photometric spectrum of the extract was revealed the highest absorbance at 407 nm, which is unique property of iron-associated heme compound. Commercial blood-forming medicine is a heme-iron originated from horse spleen ferrichin. To determine the commercial potential of the recombinant *E. coli*-synthesized iron-associated heme, mice were fed the iron-free provender mixed with the heme-producing *E. coli* biomass. The average body weight reduction of mice fed non-iron provender was 2.3% while that of mice with heme-producing *E. coli* biomass

addition was non-detectable after 15 days. The blood hemoglobin and iron contents for negative control and for biomass fed mice are discussed.

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References

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